

# Biofloc technology: principles focused on potential species and the case study of Chilean river shrimp *Cryphiops caementarius*

David Antonio Ulloa Walker<sup>1</sup>, María Cristina Morales Suazo<sup>2</sup>  and Maurício Gustavo Coelho Emerenciano<sup>3</sup> 

- 1 Programa Cooperativo Doctorado en Acuicultura, Universidad de Chile, Universidad Católica del Norte, Pontificia Universidad Católica de Valparaíso, Chile, Grupo de Marcadores Inmunológicos. Laboratorio de Genética e Inmunología Molecular. Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
- 2 Universidad Católica del Norte, Facultad de Ciencias del Mar, Departamento de Acuicultura- Larrondo 1281, Coquimbo, Chile
- 3 Santa Catarina State University (UDESC), Aquaculture Laboratory (LAQ), Laguna, SC, Brazil. Present address: CSIRO Agriculture and Food, Aquaculture Program, Bribie Island Research Centre, Bribie Island, Qld, Australia

## Correspondence

María Cristina Morales Suazo, Universidad Católica del Norte, Facultad de Ciencias del Mar, Departamento de Acuicultura-Larrondo 1281, 1781421 Coquimbo, Chile. Email: mcmorale@ucn.cl

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## Abstract

The accelerated growth of aquaculture has caused environmental impacts in many countries. Examples include the use of large volumes of water, discharge of effluents with high nutrient content, the occupation of large areas, natural habitat alternation and the escape of exotic species. Biofloc technology (BFT) is an aquaculture tool that requires minimal water exchange, promotes the nutrient recycling optimizing resources and produces natural food *in situ* by forming suspended microbial aggregates in the water (bioflocs). These microorganisms provide multiple benefits such as water quality control, pathogen resistance and nutritional supplementation. Species such as *Litopenaeus vannamei* and tilapia have been successfully applied in BFT. In addition, there are also an increasing number of studies focused on alternative species with promising results. This paper describes essential aspects of biofloc technology, its application in aquaculture and the potential to extend its benefits to new aquaculture species such as Chilean river shrimp *Cryphiops caementarius*. This paper describes the future challenges of this technology as well as opportunities for its application.

**Key words:** Aquaculture, biofloc, candidate species, carbon source, Chile, microbial aggregates.

## Introduction

Biofloc technology (BFT) is a production tool for aquaculture based on zero or minimal water exchange that can reduce discharges of nutrient-rich effluents into the environment as well as the negative impacts associated with the escape of cultivated species and the spread of disease (Wasielesky *et al.* 2006; Avnimelech, 2007; Samocha *et al.* 2007). This technology has been successfully applied in freshwater fish such as tilapias (*Oreochromis aureus*, *O. niloticus*, *O. mossambicus*) (Avnimelech *et al.* 1989; Avnimelech, 1999; Brol *et al.* 2017; Verster, 2017), marine crustaceans such as *Litopenaeus vannamei* (Burford *et al.* 2004; Wasielesky *et al.* 2006; Samocha *et al.* 2007), *Penaeus monodon* (Anand *et al.* 2013, 2014, 2017) and freshwater prawn like *Macrobrachium rosenbergii* (Asaduzzaman *et al.*

2008; Crab *et al.* 2010a). This technology has been developed mainly in tropical and subtropical geographical areas that have abundant natural light for omnivorous organisms that encompass certain stages of production or the entire life cycle (Neal *et al.* 2010).

In recent years, research and scientific publications on BFT have intensified, both in species with commercial value and other candidate species that could benefit from the application of this technology. These include freshwater fish such as *Clarias gariepinus* (Putra *et al.* 2017), *Labeo rohita* (Mahanand *et al.* 2013) and *Rhamdia quelen* (Poli *et al.* 2015). Other species of commercial interest such as the ornamental fish *Carassius auratus* (Faizullah *et al.* 2015) and *Pseudotropheus saulosi* (Harini *et al.* 2016), as well as the sea cucumber *Apostichopus japonicus* (Chen *et al.* 2018b), belonging to the Phylum Echinodermata, account

for a wide range of potential species that can be cultivated with BFT.

Among the future challenges of this technology are the exploration and validation of its potential to cultivate new species that require alternative models of commercial or small-scale aquaculture production. Such approach can also be applied to vulnerable species as a tool for both stock recovery and repopulation in wild. Based on data previously collected, the river shrimp *Cryphiops caementarius* is an endemic species in Northern Chile and Southern Peru that has great commercial potential (Meruane *et al.* 2006a, b) and social impact (Acuña *et al.* 2003). In Chile, this species is classified as vulnerable in the wild species classification register (RCE, Ministerio de Medio Ambiente, 2011) and is also listed in the International Union for the Conservation of Nature (De Grave *et al.* 2013) as a species of concern. *C. caementarius* belong to the Family Palaemonidae, as same as those of the genus *Macrobrachium*, such as *M. rosenbergii*, *M. carcinus* and *M. americanum*. Omnivorous species with marked territorial behaviour are relevant for the implementation and validation of BFT.

A fundamental aspect of aquaculture is the acquisition of juvenile individuals. The process for obtaining juveniles is attainable in Chile since reproduction control exists to maintain broodstock. This premise ties into the themes of aquaculture diversification and small-scale systems, which projects the development of the aquaculture industry in the Northern part of the country while considering new productive models for technologies and species of interest for research and commercialization. As such, *C. caementarius* is considered as a candidate species for diversification and small-scale aquaculture in continental waters since reproduction control is now feasible (Morales & Meruane, 2012; Moreno *et al.* 2012; Rojas *et al.* 2012) along with juvenile production (Morales, 1997; Morales *et al.* 2006; Meruane *et al.* 2006a,b). It is therefore possible to bring this aquaculture proposal using BFT as an alternative model for sustainable and environmentally friendly aquaculture development, with a high potential to expand into arid areas of Northern Chile.

The objectives of this paper are to describe (i) the principles, essential aspects and applications of biofloc technology in aquaculture; (ii) the potential to extend BFT benefits to new candidate species such as the Northern freshwater shrimp *C. Caementarius*; and (iii) describe the opportunities and future challenges that such technology currently faces.

## History of biofloc technology

The biofloc production system was developed as an alternative to the conventional aquaculture production systems (extensive and semi-extensive) that are used in the cultivation of commercial species such as shrimp and tilapia; and/

or as a tool during early cultivation during nursery phases. The BFT was originated in the 1970s at the French Research Institute for Exploitation of the Sea (IFREMER), located in Tahiti, French Polynesia, where Gerard Cuzon was one of the pioneers and in partnership with private companies from the United States of America (Emerenciano *et al.* 2012b; Anjalee-Devi & Madhusoodana-Kurup, 2015). It was later expanded to commercial shrimp farms (e.g. in Tahiti, Sopomer farm). In the 1990s, scientific studies and commercial pilot-scale trials began at the Waddell Mariculture Center in the United States of America with penaeid shrimp led by J. Stephen Hopkins and with finfish at the Technion-Israel Institute of Technology led by Yoram Avnimelech (Emerenciano *et al.* 2013d). In the mid-2000s, two major research centres began several studies that were fundamental to the development of BFT technology in South America at Federal University of Rio Grande-FURG (Brazil) research centre led by Wilson Wasielesky and North America in the Texas A&M University (Corpus Christi Campus, USA) led by Tzachi Samocha both focused on penaeid shrimp. Thanks to the training of human resources in these institutions, various professionals spread BFT knowledge and implemented commercial farms worldwide.

There was a significant increase in number of scientific publications on the subject of biofloc technology worldwide. The number has increased from less than 10 in 2009 to more than 100 publications in 2018, with studies conducted mainly in Brazil, China, the United States of America, Mexico and India (Scopus, 2019), helping to strengthen the technology and boost the industry. Another important factor for such progress was the wide range of courses and lectures offered in both scientific and commercial events for the scientific community, academia and aquaculturists. However, despite the progress and benefits of BFT as reported by the scientific community and academia, there is still room for its commercial expansion. For example, in Indonesia, it is estimated that only 20–25% of shrimp production has occurred using biofloc technology (Thong, 2014). Among the reasons behind such scenario are the higher implementation and production costs (e.g. electricity) compared to traditional land-based systems, and the complexities in management and implementation of the technology, which requires greater technical knowledge and permanent monitoring of water quality (Avnimelech, 2015).

It is important to note that the application of biofloc technology has focused on primarily omnivorous aquatic organisms. Assessments of candidate species for BFT should include their adaptability to intensive farming conditions, the phase of their production cycle and (i) tolerance to low-medium levels of ammonia nitrogen, nitrite and suspended solids (Samocha *et al.* 2007; Baloi *et al.* 2013; Schweitzer *et al.*, 2013a,2013b; Samocha 2019); (ii) possess an adequate morphological structure that will

enable the cultivated species to graze the bioflocs properly (Kim *et al.* 2015); (iii) capacity to digest and assimilate the microbial aggregates (Azim *et al.* 2003; Avnimelech, 2006; Smith & Sanderson, 2008; Kent *et al.* 2011); and (iv) good market value. In this sense, any candidate species must meet certain basic criteria to be considered for cultivation with the use of BFT.

As of 2015, Chile began the first studies on BFT application with *Cryphiops caementarius*, an endemic freshwater shrimp, whose cultivation technology facilitates the management of juvenile production. These studies were conducted by researchers from the Aquaculture Department of Universidad Católica del Norte (UCN-Chile) (FONDEF ID15I10353, 2018). In this project, the purpose was to evaluate the technological feasibility of the Northern river shrimp culture *C. caementarius* using the basics of biofloc technology. The aquaculture development for this species in the Northern zone of Chile should consider as an environmental restriction the situation of scarcity of natural water resources, as well as the aridity condition of the territory. The technological feasibility of cultivation considers BFT, because it allows the development of cultivation systems with a reduced water exchange, with a reuse of nutrients, an adequate water quality and the formation of bioflocs, which can be used as a complementary and permanent food by the shrimp. In this way, it is possible to considering an innovative small-scale aquaculture model, with a native species, feasible to develop in arid zones where this species is distributed.

### Basics of biofloc technology

Biofloc technology is an aquaculture production system as recirculating aquaculture systems (RAS), cage farming, pens and earth ponds. It is also the basic functional unit of the system, made up of heterogeneous aggregates of organic matter comprising a wide range of microorganisms such as chemoautotrophic and heterotrophic bacteria, cyanobacteria, archaea, viruses, microalgae, yeasts and fungi, as well as invertebrates such as rotifers, protozoa, amoebas, copepods, cladocera, ostracods, annelids and nematodes, all of which may be included in bioflocs or move freely in the water column (Hargreaves, 2006; De Schryver *et al.* 2008; Avnimelech, 2009; Browdy *et al.* 2012; Monroy-Dosta *et al.* 2013; Lara *et al.* 2016; Martínez-Córdova *et al.* 2016; Ahmad *et al.* 2017; Becerril-Cortés *et al.* 2018; Sgnaulin *et al.* 2018). Other components that form parts of the bioflocs are organic matter particles such as uneaten food, faeces, remains of dead organisms, suspended exoskeletons, organic polymers and colloids that, together with microorganisms, form conglomerates of variable size from a few microns to millimetres (De Schryver *et al.* 2008; Hargreaves, 2013). The bioflocs are held together in a flexible

matrix of exo-polysaccharides (mucus) that are secreted by bacteria, as well as by the presence of filamentous microorganisms or the electrostatic attraction between the particles that compose it (Hargreaves, 2013). Additionally, typical bioflocs have irregular shapes with a fine texture that makes them easily compressible, deformable and with a porosity over 99% (Chung & Lee, 2003; Chu & Lee, 2004). Bioflocs are denser than water so they tend to sink at a relatively slow rate of 1–3 m h<sup>-1</sup> (Sears *et al.* 2006).

Among the three main roles of bioflocs are (i) water quality control, (ii) the constitution of a food supplement for cultivated species and (iii) microbial competition with pathogens (Hargreaves, 2013). These advantages have been reported by different researchers who emphasize different aspects, mainly in shrimp farming. Several studies have therefore reported that BFT promoted higher reproductive outcomes in penaeid shrimp (Emerenciano *et al.* 2012b, 2013a,b) and freshwater fish (Ekasari *et al.* 2013; Ekasari *et al.* 2016), improvements on fish larvae (Ekasari *et al.* 2015; García-Ríos *et al.* 2019) and shrimp larvae performance (De Lorenzo *et al.* 2016) as a result of better sanitary conditions and enhanced immune systems (Wasielesky *et al.* 2006; Xu & Pan, 2013). The zero or limited water exchange also improve the farm biosecurity and reduce the spread of diseases (McIntosh *et al.* 2000; Wasielesky *et al.* 2006; Crab, 2010; Moss *et al.* 2012). As such, microbial communities associated with BFT not only recycle the nitrogen compounds in water but also protect against pathogens such as AHPND in shrimp (Hostins *et al.* 2019) and ectoparasites in tilapia (Emerenciano *et al.* 2013d) while also enhancing feed utilization and the growth of cultured organisms (Kim *et al.* 2014).

### Maintenance of water quality and Carbon: Nitrogen (C:N) ratio

Water quality control occurs primarily through the removal of toxic forms of nitrogen such as ammonium and nitrite (Asaduzzaman *et al.* 2008; Ray *et al.* 2010) by microbial communities present in culture ecosystems (Avnimelech, 1999) such as heterotrophic, photoautotrophic and chemoautotrophic organisms (Ebeling *et al.* 2006). The proportion and predominance of some of these groups of microorganisms are due to the interaction of different biotic and abiotic factors, exhibiting an ecological succession over time that is part of the biofloc formation and development process (Yusoff *et al.* 2002; Martínez-Córdova *et al.*, 2015).

A practical way to distinguish the evolution of microbial composition in BFT-based culture system is based on the colour of the medium. When a culture is started from zero (clear water), normally microalgae predominate shortly first giving the water a green and brown colour. Due to high water transparency, light penetration and nutrient

availability, this first stage is predominantly photoautotrophic which should not last more than three weeks. Through the application of external carbon sources and exogenous bacteria (known as probiotics or bioremediators), changes in colour might occur over time, indicating that heterotrophic bacteria are predominating over microalgae (Hargreaves, 2013), for example, from green to brown in freshwater conditions or in specific marine conditions dominated by green chlorophytes. The heterotrophic bacteria use the carbon available as energy source and ammonia nitrogen as nitrogen for protein synthesis (Hargreaves, 2013). Based on this considering that the efficiency of nitrogen uptake by the bacteria is approximately 40% (Rittmann & McCarty, 2001), such systems can be intervened by stimulating heterotrophic bacteria in the medium with a C:N ratio >10:1 (Emerenciano *et al.* 2017), which in most cases requires the application of external carbon sources to achieve it.

In addition, after 6–8 weeks the stabilization of nitrifying bacteria also occurs (Emerenciano *et al.* 2017) and this phenomenon can be easily identified by means of nitrification curves (Ebeling *et al.* 2006). At this point, the exogenous carbon sources addition should be reduced or even eliminated to avoid an excess of nutrient input and consequently bioflocs in the medium. This excess can lead a reduced levels of dissolved oxygen, an increase in nitrogen compounds and injuries to animal's gills (Ray *et al.* 2010; Scheveitzer *et al.* 2013a).

The monitoring and control of water quality parameters as well as the balance between carbon and nitrogen (C:N) present in the medium are crucial to the success of the stages described above (Avnimelech, 1999). Depending on this relationship, a correct microbial succession is supported with the growth of heterotrophic, nitrifying and/or microalgae bacteria by converting ammonium nitrogen into microbial protein, nitrate or microalgae biomass, respectively (Avnimelech, 1999; Ebeling *et al.* 2006). The conversion of ammonium to microbial protein consumes less dissolved oxygen compared to the requirements for nitrification (Avnimelech, 2006; Ebeling *et al.* 2006). The growth rate and yield of microbial biomass per unit of heterotrophic bacteria are ten times higher than nitrifying bacteria (Hargreaves, 2006). Microalgae have an equally important role not only because their nutritional role but also because they are efficient in the removal of phosphorus and nitrate while also can partially contribute to the removal of ammonium (Collazos-Lasso & Arias-Castellanos, 2015). The microbial community associated with BFT is not only able to treat the water from nitrogenous wastes, but also improve fish/shrimp feed utilization and growth (Azim & Little, 2008; Kim *et al.* 2014) for those species with adequate morphological structure enabling to graze the microbial aggregates (Kim *et al.* 2015).

Another significant finding regarding the C:N ratio is that shrimp and teleostean fish are ammonotelic organisms that, on average, assimilate about 20–30% of the nitrogen present in food (Jiang *et al.* 2000; Avnimelech & Ritvo, 2003). The remaining fraction is lost as nitrogen waste, mainly as ammonium through gill excretion, which is a compound that is soluble and toxic at very low concentrations (Emerenciano *et al.* 2017). Thus, one of the main factors controlling the level of nitrogenous wastes in the culture environment is the C:N ratio. This control is much more evident, especially in the early stages of cultivation where nitrifying communities are not yet fully established. This relationship depends mostly on the proportion of these elements in the formulated feed and on external carbon sources. In general, artificial feeds that contain 30–45% protein represent a C:N ratio of approximately 11 to 6:1. For example, a balanced feed containing 35% protein has a C:N ratio of about 8:1. In order to support the development of heterotrophic bacteria that provide a faster and more stable ammonium removal pathway, it is necessary to intervene in culture medium by applying an external carbon sources (Deng *et al.* 2018), elevating the C:N ratio to at least 10:1, or even as high as 20:1 (Hargreaves, 2013). Such differences in ratio will depend on stock density applied, period (days) of culture, selection of species, among others. This procedure promotes the natural productivity (Crab *et al.* 2010a), and due to an increase in microbial biomass, the oxygen demand also increases proportionally. In this sense, the dissolved oxygen and pH levels tend to decrease, and such factors need to be consider and controlled since they can cause yields constraints (De Schryver *et al.* 2008).

The selection of external carbon sources needs to address some requirements such as easy access in the local market, low costs and standardized formats (liquid, powder, flour). Furthermore, they must be easily soluble or miscible in water, labile and with good bioavailability for bacterial activity. Adequate palatability and digestibility should also be considered, but a fundamental criterion is a high proportion of carbon (over 30 % on average) with a minimum amount of nitrogen. Therefore, sources rich in carbohydrates are the most desirable.

The application of various external carbon sources influences water quality, animal behaviour, and the quality and composition of biofloc (Crab *et al.* 2010a; Monroy-Dosta *et al.* 2013; Wei *et al.* 2016). The most commonly used carbon sources are mainly derived from industrial processes or waste by-products. The sources most commonly used in a variety of studies associated with BFT are sucrose, dextrose (a simple carbohydrate obtained from starch), glycerol (by-product of biodiesel), glucose, acetate, starch, cellulose, molasses (mainly as sucrose), wheat flour, cornflour, rice bran (cellulose) and tapioca (derived from the cassava plant), among others (Table 1). The most commercially

**Table 1** Different carbon sources used in biofloc technology system in different studies worldwide

Carbon Sources	Species	References
Acetate, glucose, glycerol	<i>Macrobrachium rosenbergii</i>	Crab <i>et al.</i> (2010a)
Beet molasses	<i>Cyprinus carpio</i>	Najdegerami <i>et al.</i> (2016)
Brewery residues, cassava flour, sugarcane molasses, wheat bran	<i>Litopenaeus schmitti</i>	Fugimura <i>et al.</i> (2015)
Cellulose, sorghum	<i>Oreochromis niloticus</i>	Avnimelech <i>et al.</i> (1989)
Corn flour	<i>Oreochromis niloticus</i> , <i>O. aureus</i>	Milstein <i>et al.</i> (2001)
Corn meal	<i>Oreochromis niloticus</i> , <i>O. mossambicus</i> , <i>O. andersonii</i>	Day <i>et al.</i> (2016)
De-oiled oil palm kernel meal	<i>Litopenaeus vannamei</i>	Syamala <i>et al.</i> (2017)
Dextrose	<i>Litopenaeus vannamei</i>	Gaona <i>et al.</i> (2011), De Lorenzo <i>et al.</i> (2016)
Dextrose, molasses	<i>Litopenaeus vannamei</i>	Suita (2009), Suita <i>et al.</i> (2015)
Glucose	<i>Oreochromis niloticus</i>	Long <i>et al.</i> (2015)
Glucose, glycerol	<i>Oreochromis sp.</i>	Ekasari <i>et al.</i> (2010)
Glucose, glycerol, starch	<i>Litopenaeus vannamei</i>	Wei <i>et al.</i> (2016)
Glycerol	<i>Clarias gariepinus</i>	Dauda <i>et al.</i> (2018a)
Glycerol, molasses, sucrose	<i>Litopenaeus vannamei</i>	Ray and Lotz (2014)
Glycerol, rice bran, sucrose	<i>Clarias gariepinus</i>	Dauda <i>et al.</i> (2017)
Longan powder (LP), poly-hydroxybutyrate-hydroxyvalerate/LP (PHBVL), Poly(butylene succinate)/LP (PBSL)	<i>Oreochromis niloticus</i>	Li <i>et al.</i> (2018a)
Molasses	<i>Farfantepenaeus brasiliensis</i>	De Souza <i>et al.</i> (2014)
Molasses	<i>Litopenaeus vannamei</i>	Burford <i>et al.</i> (2004), Samocha <i>et al.</i> (2007), Krummenauer <i>et al.</i> (2011)
Molasses	<i>Litopenaeus vannamei</i>	Godoy <i>et al.</i> (2012), Da Silva <i>et al.</i> (2013)
Molasses	<i>Litopenaeus vannamei</i>	Schweitzer <i>et al.</i> (2013a,b), Ekasari <i>et al.</i> (2014a), Maia <i>et al.</i> (2016), Xu <i>et al.</i> (2016)
Molasses	<i>Oreochromis sp.</i>	Widanarni <i>et al.</i> (2012), Ekasari <i>et al.</i> (2015), Cavalcante <i>et al.</i> (2016)
Molasses	<i>Penaeus monodon</i>	Panjaitan (2004)
Molasses cane sugar, dextrose, rice bran	<i>Litopenaeus vannamei</i>	Serra <i>et al.</i> (2015)
Molasses, coffee waste, dry moringa	<i>Carassius auratus</i>	Castro <i>et al.</i> (2016)
Molasses, coffee waste, rice brand	<i>Oreochromis niloticus</i>	Becerril-Cortés <i>et al.</i> (2018)
Molasses, rice bran	<i>Farfantepenaeus brasiliensis</i>	Emerenciano <i>et al.</i> (2012a)
Molasses, rice bran	<i>Litopenaeus vannamei</i>	Maicá <i>et al.</i> (2012), Zhao <i>et al.</i> (2016)
Molasses, rice bran	<i>Litopenaeus vannamei</i>	Vilani <i>et al.</i> (2016)
Molasses, rice powder	<i>Oreochromis niloticus</i>	Maya Gutiérrez <i>et al.</i> (2016), Castro Mejía <i>et al.</i> (2017)
Molasses, starch, wheat flour, mixture of them	<i>Litopenaeus vannamei</i>	Khanjani <i>et al.</i> (2017)
Molasses, tapioca, tapioca by-product, rice bran	<i>Litopenaeus vannamei</i>	Ekasari <i>et al.</i> (2014b), Azhar <i>et al.</i> (2016)
Molasses, wheat bran	<i>Farfantepenaeus duorarum</i>	Emerenciano <i>et al.</i> (20113a)
Molasses, wheat bran	<i>Farfantepenaeus paulensis</i>	Emerenciano <i>et al.</i> (2011)
Poly-B-hydroxybutyric acid, glucose	<i>Oreochromis niloticus</i>	Luo <i>et al.</i> (2017)
Rice bran, ground bread crumb, corn meal	<i>Oreochromis niloticus</i>	Wankanapol <i>et al.</i> (2017)
Rice flour	<i>Penaeus monodon</i>	Anand <i>et al.</i> (2013)
Rice flour, molasses	<i>Penaeus monodon</i>	Kumar <i>et al.</i> (2017)
Starch	<i>Litopenaeus vannamei</i>	Liu <i>et al.</i> (2014)
Starch	<i>Oreochromis spp.</i>	Avnimelech (2007), Crab <i>et al.</i> (2009)
Sucrose	<i>Litopenaeus vannamei</i>	Kuhn <i>et al.</i> (2010), Ray <i>et al.</i> (2011), Xu and Pan (2012, 2013, 2014)
Sucrose	<i>Marsupenaeus japonicus</i>	Zhao <i>et al.</i> (2012)
Sugar beet molasses, sugar, corn starch	<i>Cyprinus carpio</i>	Bakhshi <i>et al.</i> (2018)
Sugarcane molasses, tapioca flour, wheat flour	<i>Litopenaeus vannamei</i>	Rajkumar <i>et al.</i> (2015), Pamanna <i>et al.</i> (2017)
Tapioca flour	<i>Penaeus monodon</i>	Hari <i>et al.</i> (2004), Hari <i>et al.</i> (2006)
Tapioca starch	<i>M. rosenbergii x, O. niloticus</i>	Asaduzzaman <i>et al.</i> (2009)
Tapioca starch	<i>Macrobrachium rosenbergii</i>	Asaduzzaman <i>et al.</i> (2008), Asaduzzaman <i>et al.</i> (2010)



**Table 1** (continued)

Carbon Sources	Species	References
Tapioca, starch, plant cellulose	<i>Pelteobagrus vachelli</i>	Deng <i>et al.</i> (2018)
Tapioca, wheat, corn, sugar bagasse	<i>Labeo rohita</i>	Ahmad <i>et al.</i> (2016)
Wheat flour	<i>Apostichopus japonicus</i>	Chen <i>et al.</i> (2018a)
Wheat flour	<i>Oreochromis niloticus</i>	Azim and Little (2008)
Wheat flour	<i>Penaeus monodon</i>	Anand <i>et al.</i> (2014)
Wheat flour	<i>Penaeus semisulcatus</i>	Megahed (2010)
Wheat flour, molasses	<i>Litopenaeus vannamei</i>	Peixoto <i>et al.</i> (2018)
Wheat milling by-product, rice bran	<i>Oreochromis niloticus</i>	Mansour and Esteban (2017)

used sources are molasses and flours derived from edible plants, due to their low cost and accessibility. Molasses can be used to provide more stable control of ammonium, which may be due to the presence of secondary non-carbohydrate components such as minerals and amino acids that may contribute to the heterotrophic bacteria growth (Curtin & Lane, 1983). However, the composition of molasses may vary greatly depending on the processing technologies used, water content and sugar cane variety (OECD, 2011). In addition, some biodegradable polymers such as polyhydroxybutyrate (PHB) and other sources have recently been explored as new carbon sources for the production of bioflocs in bioreactors (Li *et al.* 2018a).

### Biofloc as a nutritional supplement

Concerning the role of bioflocs as a food supplement, many studies have reported the benefits of these aggregates by increasing feed utilization, improving feed conversion (Da Silva *et al.* 2013; Furtado *et al.* 2015), growth (Wasielesky *et al.* 2006; Azim & Little, 2008; Emerenciano *et al.* 2011; Emerenciano *et al.* 2012a) and stimulation of digestive enzyme activity (Xu & Pan, 2012; Durigon *et al.* 2019). All of these factors have been shown to optimize the animal growth and survival by improving their health, enhance the immune systems (Xu & Pan, 2013; Panigrahi *et al.* 2019a,b) and provide high antioxidant activity (Kim *et al.* 2014).

In some BFT studies, enzyme activity has been used to evaluate the response to feed consumption. Protease, amylase and lipase activity have been reported where exogenous enzymes produced by the constituent bacteria of biofloc and ingested by shrimp appear to increase the activity of endogenous enzymes (Yu *et al.* 2007; Wang 2007; Anand *et al.* 2014). Similar effects have been detected in *Cyprinus carpio* cultivated with BFT (Bakhshi *et al.* 2018). Enzyme activity helps to break down proteins, carbohydrates and other macromolecules, which facilitates digestibility and nutrient absorption by cultured shrimp (Xu & Pan, 2012). Studies conducted by Cardona *et al.* (2015a) with *L. stylirostris* in BFT showed higher levels of enzyme activity and higher gene expression for amylase and trypsin,

with a growth rate that was 4.4 times higher than that of shrimp cultivated in clear water. These results are consistent with those recorded by Anand *et al.* (2014) who observed higher amylase and protease activity in *Penaeus monodon* juveniles cultivated with a supplementary biofloc diet, which obtained higher growth rates compared to the control group.

Biofloc is a natural food source available 24 hours a day (Avnimelech, 2007). The microbial protein and lipid components are considered the main nutritional contributions (Tesser *et al.* 2019) although unknown growth factors may also occur (Emerenciano *et al.* 2012b). The cultured organisms have a permanent supply of *in situ* live food (Avnimelech 2007) in which the biological renewal rate of biofloc may last <24 hours, indicating that new bioflocs are created while the old ones are captured and mineralized (Avnimelech & Kochba, 2009). Due to constant nutrient recycling, BFT acts as a fresh source of feed supplement, optimizing the intake of balanced feed and improving the growth rates (Tacon *et al.* 2002; Burford *et al.* 2004; Ju *et al.* 2008a; Kuhn *et al.* 2010). According to Burford *et al.* (2004), the contribution in nitrogen retention derived from the natural productivity or bioflocs was between 18–29% in adult shrimp of *Litopenaeus vannamei* cultivated with biofloc technology in commercial farms in Central America.

The nutritional value of biofloc is closely related to the microbial community that composes it (Ju *et al.* 2008b; Ekasari *et al.* 2010; Widanarni *et al.* 2010). The biochemical composition is affected by several factors such as light exposure, temperature, pH, carbon sources and salinity (Maicá *et al.* 2012; Emerenciano *et al.* 2013d; Martínez-Córdova *et al.* 2015). In general, the nutritional composition of biofloc is characterized by high protein levels ranging between 14% and 50%, followed by carbohydrates and lower lipids between 1.2% and 9% (Martínez-Córdova *et al.*, 2015). Although a trend exists in the expected values of biofloc proximate analysis (e.g. crude protein and carbohydrates around 30%, lipids and ashes less than 3.0% and 30%, respectively), several other factors may contribute to diverse results such as species and feed type (protein content, feedstuff, etc.), biofloc size (Ekasari *et al.* 2014a) and

**Table 2** Proximate composition of biofloc based on published data according to bibliographic references

Species	Protein Content Food (%)	Crude Protein (%)	Carbohydrates (%)	Lipids (%)	Crude Fibre (%)	Ash (%)	References
Shrimp							
<i>Farfantepenaeus brasiliensis</i>	40	30.4	29.4	0.5	0.8	39.2	Emerenciano <i>et al.</i> (2012a)
<i>Farfantepenaeus duorarum</i>	35	28.0–30.4	18.1–22.7	0.5–0.6	3.1–3.2	35.8–39.6	Emerenciano <i>et al.</i> (2013a)
<i>Farfantepenaeus paulensis</i>		30.4		0.5	0.8	39.2	Ballester <i>et al.</i> (2010)
<i>Fenneropenaeus indicus</i>		18–23	51–62	17–22	–	4–3	Megahed and Mohamed (2014)
<i>Litopenaeus schmitti</i>		16–18	–	1.5–2.4	–	54–72	Fugimura <i>et al.</i> (2015)
<i>Litopenaeus vannamei</i>	–	35–51	–	10–15	–	21–32	Chamberlain <i>et al.</i> (2001)
<i>Litopenaeus vannamei</i>	35	31.2	–	2.6	–	28.2	Tacon <i>et al.</i> (2002)
<i>Litopenaeus vannamei</i>	25–35	31.1	23.6	0.49	–	44.8	Wasielsky <i>et al.</i> (2006)
<i>Litopenaeus vannamei</i>	35–40	26–41.9	–	1.2–2.3	–	18.3–40.7	Ju <i>et al.</i> (2008a)
<i>Litopenaeus vannamei</i>	40	30.4	–	1.9	12.4	38.9	Ju <i>et al.</i> (2008b)
<i>Litopenaeus vannamei</i>	–	49	36.4	1.13	12.6	13.4	Kuhn <i>et al.</i> (2009)
<i>Litopenaeus vannamei</i>	45	38.8	25.3	<0.1	16.2	24.7	Kuhn <i>et al.</i> (2010)
<i>Litopenaeus vannamei</i>	42.5	28.8–43.1	–	2.1–3.6	8.7–10.4	22.1–42.2	Maicá <i>et al.</i> (2012)
<i>Litopenaeus vannamei</i>	35	27.3–31.6	–	3.7–4.2	–	43.7–49.4	Xu and Pan (2012)
<i>Litopenaeus vannamei</i>	35	18.4–26.3	20.2–35.7	0.3–0.7	2.1–3.4	34.5–41.5	Emerenciano <i>et al.</i> (2013b)
<i>Litopenaeus vannamei</i>		15.7	–	1.6	–	–	Schweitzer <i>et al.</i> (2013a,b)
<i>Litopenaeus vannamei</i>	24.3–36.7	18.3–20.3	–	1.4–1.9	16.6–27.1	–	Jatobá <i>et al.</i> (2014)
<i>Litopenaeus vannamei</i>	–	50.6–53.5	–	3.8–4.0	–	7.4–7.5	Rostika (2014)
<i>Litopenaeus vannamei</i>	35	21.3–32.1	–	1.6–2.8	–	43.4–61.4	Xu and Pan (2014)
<i>Litopenaeus vannamei</i>	34.5	46–53.6	–	0.6–0.9	12.9–16.7	14.9–25	Rajkumar <i>et al.</i> (2015)
<i>Litopenaeus vannamei</i>		31.4–31.8	–	1.4–1.6	–	–	Suita <i>et al.</i> (2015)
<i>Litopenaeus vannamei</i>	42	41.2–35.5	37.7–47.6	4.2–8.5	–	12.4–15.2	Wei <i>et al.</i> (2016)
<i>Macrobrachium rosenbergii</i>	–	28–43	29–50	2.3–5.4	–	17–27	Crab <i>et al.</i> (2010b)
<i>Penaeus monodon</i>	–	12–42	–	2–8	–	22–46	Soares <i>et al.</i> (2004)
<i>Penaeus monodon</i>	–	24.3	–	3.5	3.1	32	Anand <i>et al.</i> (2014)
Fish							
<i>Carassius auratus</i>	32	21.9	20.5	1.0	–	51.4	Faizullah <i>et al.</i> (2015)
<i>Carassius auratus</i>		29.8			3.2	19.1	Zhang <i>et al.</i> (2018)
<i>Clarias gariepinus</i>		31.3–33.3		0.5–0.8	–	6.7–6.8	Dauda <i>et al.</i> (2017)
<i>Clarias gariepinus</i>		32.6–44.3		5.8–10.8	–	4.6–7.0	Dauda <i>et al.</i> (2018a)
<i>Oreochromis niloticus</i>	24–35	38	–	3.2	6	12–13	Azim and Little (2008)
<i>Oreochromis niloticus</i>	46	41.1	–	1.0	–	6.1	Long <i>et al.</i> (2015)
<i>Oreochromis niloticus</i>	35	30.2–48	–	2.0–2.5	3.9–29.1	6.7–16.5	Becerril-Cortés <i>et al.</i> (2018)
<i>Oreochromis niloticus</i>	–	28.1–35.3	–	5.1–6.7	–	–	Li <i>et al.</i> (2018a)
<i>Oreochromis sp.</i>	40	28–33	–	6–9	–	7–13	Ekasari <i>et al.</i> (2010)
<i>Oreochromis sp.</i>	–	39.7–48.1	–	12.6–24.3	3.1–4.5	25.2–28.7	Widanarni <i>et al.</i> (2012)
<i>Oreochromis sp.</i>	–	23.7–25.4	32.2–39	2.6–3.5	–	33–40.4	López-Ellías <i>et al.</i> (2015)
<i>Pseudotropheus saulosi</i>	–	20.5	21.2	0.5	–	52.4	Harini <i>et al.</i> (2016)

the C:N ratio applied. The intensity and frequency of feeding also play a role, which explains a wide range of values found in the literature (Table 2).

In regard to essential fatty acids obtained from biofloc biomass, the omega-3 contributions of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) may represent between 0.2% and 0.77%, as well as linolenic acid (ALA) with value ranging from 0.65% to 3.3%. For omega-6, arachidonic acid (ARA) may range from 0.3% to 3.55% and linoleic acid (LA) with 1.5% to 16.68% (Emerenciano *et al.* 2013d). Moreno-Arias *et al.* (2018) indicate that the amino acid and fatty acid composition of biofloc and

shrimp cultivated in BFT are independent of the composition of the formulated feed used.

Additionally, bioflocs can provide minerals such as iron, zinc, magnesium, potassium, phosphorus, calcium and sodium (Rajkumar *et al.* 2015). It has also been known to contain essential amino acids such as leucine, valine, isoleucine, phenylalanine, threonine, histidine and tryptophan (Kuhn *et al.* 2010; Emerenciano *et al.* 2013a) with limited amounts of arginine, cysteine and methionine (Ju *et al.* 2008a). A study carried out by Ekasari *et al.* (2014a) reported deficiencies in the biofloc based on the essential amino acid index (EAAI) for *Litopenaeus vannamei*, and

determined that the limited amino acids are arginine, and to a lesser extent, leucine and methionine, while those for *Oreochromis niloticus* are methionine, arginine and lysine. Meanwhile, Ju *et al.* (2008a) showed that the concentration of free amino acids such as alanine, glutamate, arginine and glycine that would act as attractants in shrimp diets (Nunes *et al.* 2006) is present in biofloc. On the other hand, the vitamins reported in the biofloc analysis are niacin, thiamine (B<sub>1</sub>), riboflavin, vitamin B<sub>12</sub> and vitamin E (Emerenciano *et al.* 2013d); however, it can be deficient in vitamin C (Crab *et al.* 2012). Besides, several bioactive components such as carotenoids, chlorophylls, polysaccharides, phytosterols, bromophenols, taurine and liposoluble vitamins were reported in BFT (Ju *et al.* 2008a). Furthermore, some researchers have suggested the existence of unknown or unidentified growth-promoting factors that would explain higher growth rates (Ju *et al.* 2008a; Kuhn *et al.* 2010) compared to conventional systems.

## Biofloc production alternatives

### Biofloc *in situ*

This form of biofloc production is the most common and based on the direct participation of cultivated aquatic organisms in the nitrogen transformation and recycling chain. In these systems, the fish or shrimp are capable of consuming and assimilating the balanced feed while also generating nitrogenous wastes, which together with the carbon available in the environment, provide nutrient sources for the microbial community. These combined factors allow for the production of new protein biomass that will then be available for consumption in the form of bioflocs.

This continuous sequence of biotransformations enables the recycling and reuse of the nitrogen present in the protein of the balanced feed. Da Silva *et al.* (2013) determined in *L. vannamei* that 39.1% from the nitrogen incorporated as shrimp feed and molasses was absorbed by the shrimp raised in biofloc system. In order to evaluate the protein content of biofloc for new tissue formation in cultivated organisms, a series of tools have been used such as nitrogen stable isotopes ( $\delta^{15}\text{N}$ ). Evaluations carried out on pelletized feed, biofloc and the muscle of *Oreochromis niloticus* confirmed that high levels of  $\delta^{15}\text{N}$  in fish muscle came from bioflocs (Avnimelech & Kochba, 2009), suggesting that 48% of the fish's nitrogen comes from the microbial aggregates. In productive terms, this means that the protein conversion efficiency goes from 4:1 in a conventional system to 2:1 in a BFT system (Avnimelech, 2009). A study conducted by Burford *et al.* (2004) used nitrogen isotopes ( $\delta^{15}\text{N}$ ) and concluded that the estimated proportion of nitrogen retention in *Litopenaeus vannamei* in biofloc was between 18% and 29%. Meanwhile, Cardona *et al.* (2015a) using naturally occurring stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) and

carbon ( $\delta^{13}\text{C}$ ) concluded that in juvenile *L. stylirostris*, 37% to 40% of the nutrients used for new tissue formation came from natural productivity (bioflocs) and also stimulated the digestive enzyme activity and improved the growth performance. Additionally, Suita *et al.* (2015) evaluated the biofloc contribution in post-larvae of *L. vannamei* using  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , highlighting the positive effect on the quality of organisms and water presumably because of variations on the microbial community, resulting in a superior growth performance of *L. vannamei* when cultured in BFT systems.

The maintenance and continuity of biofloc *in situ* are based on the ability of cultivated organisms (e.g. fish or crustacea) to form part of the trophic chain by capturing and consuming the bioflocs. Initially, the consumption of balanced feed and the excretion of nitrogenous wastes enable the continuous production of new bioflocs. However, in order to accelerate the starting point in a new process of development of biofloc, an inoculum of a pre-existing mature biofloc can be used (Krummenaeur *et al.* 2014; Martins *et al.* 2014; Thong, 2014). By the other hand, a new biofloc can be started from scratch, and in this case, it is necessary to know the characteristics of the water and then determine the fertilizers and appropriate doses of nutrients that will provide the necessary C:N ratio and the regulation of the alkalinity, pH and other parameters, if necessary.

### Biofloc *ex situ*

This form of production is done in units specially designed for the production of the bioflocs. Some of these units are known as sequencing batch reactors (SBR), whose characteristic is that they work independently or in the absence of cultivated species (De Schryver & Verstraete, 2009; Kuhn *et al.* 2009, 2010; Ruan *et al.* 2011; Luo *et al.* 2013). The levels of total suspended solids in SBR are higher compared to *in situ* production systems, since the absence of the target crop species can intensify production. The limits are therefore only based on the bacteria inside the bioreactors.

These devices are considered as 'biofloc factories', which allow for the continuous and independent production of bioflocs. These aggregates are then added to the production systems and serve as feed for fish and shrimp. In some cases, effluents from other aquaculture systems (such as RAS) are used and considered as a nutrient source for the *ex situ* production (Kuhn *et al.* 2010; Santaella *et al.* 2018). They are presented as an environmentally friendly way to recycle nutrients and to produce high-quality natural food (Emerenciano *et al.* 2013d). In these cases, attempts are made to take advantage of the nutritional and immunological properties of the aggregates (Kuhn *et al.* 2009; Martínez-Córdova *et al.* 2016), or for technical reasons,



since *ex situ* production offers advantages by independently producing bioflocs and would allow better control over the nutritional profile of the aggregates (Crab *et al.* 2010a).

### Biofloc as a feed ingredient

An alternative application is the use of biofloc as a balanced feed ingredient, either directly produced in reactors (*ex situ*) or collected as excess from decanting ponds or clarifiers associated with aquaculture production units with BFT (*in situ*). Once collected, the biofloc can be decanted or filtered into a small diameter mesh (e.g. 10 µm), centrifuged, dried and converted into a fine powder (Kheti *et al.* 2017). Another mechanism is through a freeze-drying process, in which biofloc samples are immediately placed in a freezer at  $-80^{\circ}\text{C}$  and then processed in freeze-drying equipment (Arias-Moscoso *et al.* 2016). While this process provides better maintenance of the biochemical properties of the bioflocs, the high cost may be a limiting factor.

Dry or freeze-dried biofloc can be used as an ingredient of the balanced feed and be supplied as part of the diet, providing a source of protein that acts as an alternative of replacement to fish meal or other protein sources while taking advantage of its nutritional qualities (Ju *et al.* 2008b; Kuhn *et al.* 2009; Bauer *et al.* 2012; Anand *et al.* 2014; Kheti *et al.* 2017; Lee *et al.* 2017). A study by Shao *et al.* (2017) showed that a 15% substitution of fish meal with biofloc obtained similar results in shrimp growth compared to the control group without replacement, which is equivalent to the optimal levels of replacement found by Valle *et al.* (2015). In studies done by Anand *et al.* (2017), it was concluded that adding 4% to 8% biofloc supplementation to the diet in *Penaeus monodon* produced immunomodulatory effects and improvements in the physiological condition of shrimp. This field of research has high potential because the nutritional properties of the aggregates can be varied and optimized according to the form or production method of the bioflocs.

### Ability of cultivated organisms to graze the biofloc

At present, the most cultivated species in BFT have common characteristics such as morphologically specialized structures and the ability to graze the suspended bioflocs. In this sense, tilapia are one of the most successfully cultivated species in BFT. Tilapia possess structures called microbranchiospines (Beveridge *et al.* 1988) that allow filter the water and capture the bioflocs (López Pérez *et al.* 2013; Verster, 2017). Studies by Smith and Sanderson (2008) show that suspended particles in the medium that pass through the oropharyngeal cavity of tilapia are captured by a viscous and transparent mucous secretion, which produces an aerosol filtration mechanism. Tilapia use this

function to capture smaller particles, ranging from a few microns to millimetres in diameter. Therefore, filtration by sieving through the gill rakers along with mucus is the mechanisms involved in capturing the bioflocs suspended in the medium, which explains the favourable responses of tilapia when grown with BFT.

The most commercially produced shrimp is *L. vannamei*. In studies carried out by Kent *et al.* (2011) based on an examination of setae from third maxillipeds with electron microscopy, they suggested that juveniles are capable of selecting and consuming suspended food particles approximately 10 µm in diameter using these net-like setae. With such structures, these shrimp can capture diatoms such as *Thalassiosira* and *Amphiprora*, whose sizes are approximately 10 µm. This ability would explain the high adaptation to suspended biofloc systems.

Structures that can efficiently capture particles have also been documented for other species. Such structures in freshwater prawn *Macrobrachium rosenbergii* can capture particles between 250 and 1200 µm (Barros & Valenti, 2003). For silver carp *Hypophthalmichthys molitrix*, Schroeder (1978) reported the separation of the gill rakers was between 20 and 50 µm and enables to capture biofloc. Odum (1968) reported that *Mugil cephalus* could capture particles smaller than 10 µm. Research has been conducted in recent years to evaluate the adaptation of these two fish species in systems based on biofloc technology (Zhao *et al.* 2014; Vinatea *et al.* 2018).

### Aquaculture species studied with bft

#### A review update

Research on shrimp has focused on species of commercial and/or social interest from the time they were conducted. These species include *Fenneropenaeus merguensis* (Aquacop, 1975), *L. vannamei* (McIntosh, 2000), *Penaeus monodon* (Hari *et al.* 2006), *Farfantepenaeus paulensis* (Emerenciano *et al.* 2007) and *Litopenaeus setiferus* (Emerenciano *et al.* 2009). Subsequently, biofloc technology has been extended to other species of the Family Penaeidae, such as *Penaeus semisulcatus* (Megahed, 2010), *F. brasiliensis* (Emerenciano *et al.* 2012a), *F. duorarum* (Emerenciano *et al.* 2013a,c), *L. stylirostris* (Emerenciano *et al.* 2012b), *L. schmitti* (Fugimura *et al.* 2015), *Marsupenaeus japonicus* (Zhao *et al.* 2012), *Fenneropenaeus indicus* (Megahed & Mohamed, 2014), *F. chinensis* (Kim *et al.* 2015) and *Metapenaeus monoceros* (Kaya *et al.* 2019). As for freshwater species, the prawn *Macrobrachium rosenbergii* is the only species of the Family Palaemonidae about which scientific publications associated with BFT have been published, making its commercial application known. To summarize, publications on crustaceans (Table 3) have been developed in three families: *Artemiidae*, *Palaemonidae* and

**Table 3** BFT applications in different crustaceans species based on data published according to bibliographic references

Family	Species	Historical and chronological bibliographical reference
Artemiidae	<i>Artemia franciscana</i>	Crab et al. (2010b), Nguyen et al. (2013), Ronald et al. (2014)
Palaemonidae	<i>Macrobrachium rosenbergii</i>	Asaduzzaman et al. (2008), Crab et al. (2010a), Prajith (2011), Pérez-Fuentes et al. (2013), Pérez-Rostro et al. (2014), Ballester et al. (2017), Miao et al. (2017), Negrini et al. (2017)
Penaeidae	<i>Farfantepenaeus brasiliensis</i>	De Souza et al. (2012), Emerenciano et al. (2012a), De Souza et al. (2014), Hostins et al. (2015), Magaña-Gallegos et al. (2018)
	<i>Farfantepenaeus duorarum</i>	Emerenciano et al. (2013a)
	<i>Farfantepenaeus paulensis</i>	Emerenciano et al. (2007), Ballester et al. (2010), Emerenciano et al. (2011)
	<i>Fenneropenaeus chinensis</i>	Kim et al. (2015), Kim et al. (2015)
	<i>Fenneropenaeus indicus</i>	Megahed and Mohamed (2014), Effendy et al. (2016), Megahed et al. (2018)
	<i>Litopenaeus schmitti</i>	Fugimura et al. (2015)
	<i>Litopenaeus setiferus</i>	Emerenciano et al. (2009)
	<i>Litopenaeus stylirostris</i>	Emerenciano et al. (2012b), Cardona et al. (2015a), Cardona et al. (2015b), Cardona et al. (2016a), Cardona et al. (2016b)
	<i>Litopenaeus vannamei</i>	McIntosh (2000), Tacon et al. (2002), Burford et al. (2004), Hari et al. (2006), Wasielesky et al. (2006), Samocha et al. (2007), Ju et al. (2008a), Mishra et al. (2008), Kuhn et al. (2009), Suita (2009), Kuhn et al. (2010), Krummenauer et al. (2011), Maicá et al. (2012), Xu and Pan (2012), Emerenciano et al. (2013b), Ekasari et al. (2014b), Jatobá et al. (2014), Liu et al. (2014), Rostika and Sudaryono (2014), Rajkumar et al. (2015), Serra et al. (2015), Maia et al. (2016), Vilani et al. (2016), Wei et al. (2016), Xu et al. (2016), Panigrahi et al. (2018)
	<i>Marsupenaeus japonicus</i>	Zhao et al. (2012)
	<i>Metapenaeus monoceros</i>	Kaya et al. (2019)
<i>Penaeus merguensis</i>	Aquacop (1975)	
<i>Penaeus monodon</i>	Hari et al. (2004), Panjaitan (2004), Soares et al. (2004), Hari et al. (2006), Arnold et al. (2009), Anand et al. (2013), Anand et al. (2014), Anand et al. (2017), Kumar et al. (2017)	
<i>Penaeus semisulcatus</i>	Megahed (2010)	

*Penaeidae* with a total of 16 species representing the majority (14) of the Family *Penaeidae*.

Several studies based on BFT have been developed on freshwater fish species of the Family *Cichlidae*, including *Oreochromis aureus* (Avnimelech et al. 1989), *O. mossambicus* (Avnimelech, 2007) and *O. niloticus* (Azim & Little, 2008; Brol et al. 2017). Other fish groups of commercial importance that have been the subject of scientific literature are those belonging to the Family *Cyprinidae*, notably the species *Tinca tinca* (Carbó & Celades, 2010), *Carassius auratus* (Wang et al. 2015), and *Cyprinus carpio* (Najdegerami et al. 2016). There were also studies done on two of India's major carp *Catla catla* (Prajith, 2011) and *Labeo rohita* (Mahanand et al. 2013). Similarly, studies have been done on three catfish species, the American catfish *Ictalurus punctatus* (Green, 2010), the South American catfish *Rhamdia quelen* (Poli et al. 2015) and the African catfish *Clarias gariepinus* (Yusuf et al. 2015), belonging to the families *Ictaluridae*, *Heptateridae* and *Clariidae*, respectively. In addition to these studies, research is also being done on native fish from South America, such as *Colossoma macropomum* (Itani, 2010), *Piaractus brachypomus* (Poleo et al. 2011) and *Brycon orbignyanus* (Sgnaulin et al. 2018), belonging to the Family *Characidae*. Also added are the fish *Arapaima gigas*, belonging to the Family *Arapaimidae* (Maravi, 2009) and *Prochilodus magdalenae*, belonging to

the Family *Prochilodontidae* (Roa-Lázaro et al. 2017). As for ornamental fish species, studies have been done on *Poecilia reticulata* (Sreedevi & Ramasubramanian, 2011), *Scatophagus argus* (Liu et al. 2014), *Carassius auratus* (Faizullah et al. 2015), *Pseudotropheus saulosi* (Harini et al. 2016), *Xiphophorus maculatus* (Boaventura, 2016) and *Puntius conchonius* (De Lara et al. 2017). There are also studies as recently as 2018 that have been conducted on *Anguilla spp* (glass eels) (Sukardi et al. 2018) and *Anguilla marmorata* (Li et al. 2018b).

In marine fish species, BFT improved immune functions and reduces stress in *Paralichthys olivaceus* juveniles (Kim et al. 2018). Ekasari et al. (2014a) evaluated the effects of biofloc in different trophic levels including *Oreochromis niloticus* (*Chordata*), *Litopenaeus vannamei* (*Arthropoda*) and *Perna viridis* (*Mollusca*). Another example of this is a study published on *Apostichopus japonicus* (Chen et al. 2018a) or sea cucumber, belonging to the Phylum *Echinodermata*, further extending the range of potential species that can be cultivated with biofloc (Table 4).

Additionally, studies have been conducted that combine the cultivation of two or more species such as *Macrobrachium rosenbergii* and *Oreochromis niloticus* (Asaduzzaman et al. 2009) as well as in cultures that integrate noncompeting species such as *Aristichthys nobilis*,

**Table 4** BFT applications in fish, sea cucumber and green mussel based on data published according to bibliographic references

Family	Species	Historical and chronological bibliographical reference
Anguillidae	<i>Anguilla</i> spp.	Li <i>et al.</i> (2018b), Sukardi <i>et al.</i> (2018)
Arapaimidae	<i>Arapaima gigas</i>	Maravi (2009)
Atherinopsidae	<i>Chirostoma jordani</i>	Castro-Mejía <i>et al.</i> (2016)
Bagridae	<i>Pelteobagrus vachelli</i>	Deng <i>et al.</i> (2018)
Characidae	<i>Brycon orbignyanus</i>	Sgnaulin <i>et al.</i> (2018)
	<i>Colossoma macropomum</i>	Itani <i>et al.</i> (2010)
Cichlidae	<i>Piaractus brachypomus</i>	Poleo <i>et al.</i> (2011), Abad <i>et al.</i> (2014), Chaverra <i>et al.</i> (2017)
	<i>Etoplus suratensis</i>	Thilakan <i>et al.</i> (2019)
	<i>Oreochromis aureus</i> , <i>O. niloticus</i> , <i>O. mossambicus</i> , <i>Hibridos</i>	Avnimelech and Mokady (1988), Avnimelech <i>et al.</i> (1989), Avnimelech (1999), Milstein <i>et al.</i> (2001), Avnimelech (2007), Azim and Little (2008), Crab <i>et al.</i> (2009), Widanarni <i>et al.</i> (2012), Ekasari <i>et al.</i> (2014a), Ekasari <i>et al.</i> (2015), Long <i>et al.</i> (2015), Cavalcante <i>et al.</i> (2016), Mansour and Esteban (2017), Verster (2017), Li <i>et al.</i> (2018a) Day <i>et al.</i> (2016)
	<i>Oreochromis niloticus</i> , <i>O. mossambicus</i> , <i>O. andersonii</i>	
	<i>Poecilia reticulata</i>	Sreedevi and Ramasubramanian (2011)
	<i>Pseudotropheus saulosi</i>	Harini <i>et al.</i> (2016)
	<i>Clarias gariepinus</i>	Abu Bakar <i>et al.</i> (2015), Yusuf <i>et al.</i> (2015), Hapsari (2016), Rostika <i>et al.</i> (2016), Dauda <i>et al.</i> (2017), Putra <i>et al.</i> (2017), Dauda <i>et al.</i> (2018a), Fauji <i>et al.</i> (2018), Romano <i>et al.</i> (2018)
Cyprinidae	<i>Carassius auratus</i>	Faizullah <i>et al.</i> (2015), Wang <i>et al.</i> (2015), Castro <i>et al.</i> (2016), Zhang <i>et al.</i> (2018)
	<i>Catla Catla</i>	Prajith (2011)
	<i>Cyprinus carpio</i>	Sarker (2015), Najdegerami <i>et al.</i> (2016), Bakhshi <i>et al.</i> (2018)
	<i>Labeo rohita</i>	Prajith (2011), Mahanand <i>et al.</i> (2013), Sangotra <i>et al.</i> (2015), Ahmad <i>et al.</i> (2016), Kamilya <i>et al.</i> (2017), Kheti <i>et al.</i> (2017)
	<i>Labeo victorinus</i>	Magondu <i>et al.</i> (2013)
	<i>Pimephales promelas</i>	Park <i>et al.</i> (2017)
	<i>Puntius conchoniuis</i>	De Lara <i>et al.</i> (2017)
Heptapteridae	<i>Tinca tinca</i>	Carbó and Celades (2010), Vinatea <i>et al.</i> (2018)
	<i>Rhamdia quelen</i>	Poli <i>et al.</i> (2015), Pereira <i>et al.</i> (2016), Rocha <i>et al.</i> (2017)
Ictaluridae	<i>Ictalurus punctatus</i>	Green (2010), Schrader <i>et al.</i> (2011), Green and Schrader (2015), Green and McEntire (2017)
Moronidae	<i>Morone chrysops</i> and <i>M. saxatilis</i> ( <i>híbrido</i> )	Green <i>et al.</i> (2017)
Mugilidae	<i>Liza carinata</i>	Magdy <i>et al.</i> (2016)
	<i>Mugil cephalus</i>	Vinatea <i>et al.</i> (2018)
Paralichthyidae	<i>Paralichthys olivaceus</i>	Kim <i>et al.</i> (2018)
Pangasiidae	<i>Pangasianodon hypophthalmus</i>	Nguyen <i>et al.</i> (2017)
Poeciliidae	<i>Xiphophorus maculatus</i>	Boaventura (2016)
Prochilodontidae	<i>Prochilodus magdalenae</i>	Atencio <i>et al.</i> (2015), Roa-Lázaro <i>et al.</i> (2017)
Scatophagidae	<i>Scatophagus argus</i>	Liu <i>et al.</i> (2014), Khanh <i>et al.</i> (2018)
ECHINODERMATA		
Stichopodidae	<i>Apostichopus japonicus</i>	Chen <i>et al.</i> (2018a, 2018b, 2018c)
MOLLUSCA		
Mytilidae	<i>Perna Viridis</i>	Ekasari <i>et al.</i> (2014a)

*Hypophthalmichthys molitrix* and *Cyprinus carpio* from China (Zhao *et al.* 2014) (Table 5).

#### Candidate species: the case of river shrimp *Cryphiops caementarius* in Chile

*Cryphiops caementarius* (Molina, 1782) (Decapoda: Palaemonidae), known locally in Chile as the Northern river shrimp, is the most commercially important freshwater crustacean of Northern Chile and Southern Peru, with a

restricted geographical distribution between 10°S and 32° 55'S (Bahamonde & Vila, 1971). Due to its high commercial value as well as its high social and economic importance in Chile and Peru, this palaemonid has been subjected to excessive exploitation for years, impacting its natural populations throughout the Chilean territory (Meruane *et al.* 2006b). It is reported as a species in danger of extinction in Chile's Valparaíso and Metropolitan Regions, and vulnerable for the rest of its distribution (Jara *et al.*, 2006).

**Table 5** BFT applications in polycultures or integrated multitrophic aquaculture system (IMTA) based on data published according to bibliographic references

Polycultures-IMTA	Species	Historical and chronological bibliographical reference
Penaeidae	<i>Litopenaeus vannamei</i> +	Legarda
Mugilidae	<i>Mugil curema</i>	<i>et al.</i> (2019)
Penaeidae	<i>Litopenaeus vannamei</i> +	Poli
Cichlidae	<i>Oreochromis niloticus</i> + ( <i>Sarcocornia ambigua</i> )	<i>et al.</i> (2019)
Cichlidae	<i>Clarias gariepinus</i> +	Dauda
Cyprinidae	<i>Hypsibarbus wetmorei</i> ♂ + <i>Barboides gonionotus</i> ♀	<i>et al.</i> (2018b)
Palaemonidae	<i>Macrobrachium rosenbergii</i> +	Asaduzzaman
Cichlidae	<i>Oreochromis niloticus</i>	<i>et al.</i> (2009), Reinoso (2016)
Characidae	<i>Piaractus brachypomus</i> +	Bru (2016)
Cichlidae	<i>Oreochromis niloticus</i>	
Penaeidae	<i>Litopenaeus vannamei</i> +	Liu
Scatophagidae	<i>Scotophagus argus</i>	<i>et al.</i> (2014)
Cyprinidae	<i>Aristichthys nobilis</i> + <i>Hypophthalmichthys molitrix</i> + <i>Cyprinus carpio</i>	Zhao <i>et al.</i> (2014)
Palaemonidae	<i>Macrobrachium rosenbergii</i> +	Prajith (2011)
Cyprinidae	<i>Catla catla</i> + <i>Labeo rohita</i>	

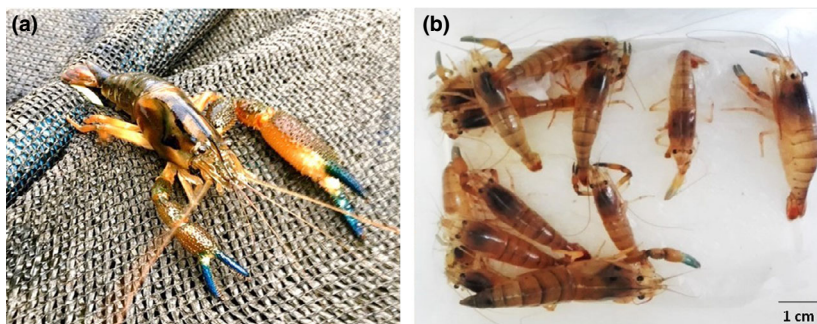
The artificial production of *C. caementarius* juveniles has been investigated for several years by different authors (Rivera & Meruane 1987; Morales, 1997; Morales *et al.*, 2006; Meruane *et al.*, 2006b), and its commercial escalation has only been achieved in the last 12 years (Morales *et al.*, 2006; Meruane *et al.*, 2006a; Morales & Meruane, 2012). Defining biological factors such as the duration of its larval development cycle, which includes 18 stages of zoea (Morales *et al.*, 2006), in addition to the development of operational aspects such as the standardization of feeding protocols and the use of indicators of larval condition has been key to finalizing the validation of results and the consolidation of culture technology (Morales *et al.*, 2006;

Meruane *et al.*, 2006b; Morales & Meruane, 2012). *C. caementarius* completes larval development at 25 °C of temperature and salinities between 15 and 20 psu (Rivera & Meruane 1987). This species has a larval stage that naturally occurs in estuaries or the sea, while for metamorphosis freshwater is required.

Chile currently produces juveniles of *C. caementarius* on experimental scale. The reproduction in captivity maintains the stock according to different river's, and the juvenile maintenance is carried out until reach 10 mm cephalothoracic length (CL) size. Recently, different studies have been initiated in the nursery phase up to market size using BFT technology.

In this sense, as a contribution to aquaculture diversification in Chile, the Universidad Católica del Norte in Coquimbo through a project financed with funds from the Ministry of Education of Chile (FONDEF ID15I10353, 2018). In 2015 began preliminary studies on the development of biofloc technology applied to river shrimp (Fig. 1). The main updated result in the cited project corresponds to the work with specimens of *C. caementarius* with an average weight of  $9.89 \pm 2.82$  g. (density of  $300 \text{ gm}^{-2}$ ), use of food formulated with 57.73% protein and a feed ration at a rate of 5% of the total shrimp biomass per pond. At 60 days of experimentation, the highest average survival was obtained in the biofloc system (93%) compared to the control systems with clear water (87%). Likewise, a higher growth level of 5.96% was obtained. The general water quality recorded in the experimental biofloc systems was  $25 \text{ mg L}^{-1}$  of VF; 0.5 to  $1.0 \text{ mg L}^{-1}$  TAN; 0.03 to  $1.0 \text{ mg L}^{-1}$   $\text{NO}_2^-$ -N; 7.0 to  $8.6 \text{ mg L}^{-1}$ ,  $\text{NO}_3^-$ -N; 5 to  $8 \text{ mg L}^{-1}$  OD; 228 to  $295 \text{ mg of CaCO}_3\text{L}^{-1}$ ; 7.6 to 8.6 pH and  $23 \pm 2^\circ\text{C}$  (FONDEF ID15I10353, 2018). Values considered appropriate for the species studied (Meruane *et al.* 2006a,b).

Previous studies showed that commercial-scale cultivation of this species would contribute to sustainable production by reducing pressure on natural populations (Meruane *et al.* 2006a). The commercial production of *C. caementarius* larvae and juveniles under captive conditions has resulted in greater size uniformity, survival and growth

**Figure 1** Male specimen (a) and first shrimps of *Cryphiops caementarius* (b), produced with BFT in Chile (FONDEF ID15I10353, 2018)



during production cycles in tanks (Morales *et al.* 2006). This species is characterized by its territorial behaviour and omnivorous eating habits that, in the natural environment, include organic matter, aquatic insects, aquatic plants and other small organisms such as fish larvae and crustaceans (Meruane *et al.* 2006a).

Initial results indicate the BFT as an alternative that could be implemented in arid areas such as Northern Chile for *C. caementarius* grow-out (Meruane *et al.* 2006b). Such species is one of the main natural freshwater resources traded in the national market and is considered a gourmet resource in the local market. *C. caementarius* was considered among twenty Chilean as the one with the potential for aquaculture production implemented in arid zones. Initial results up to commercial size (40 to 45 gr) demonstrated satisfied survival, growth and water savings (CORFO 2016). In this sense, the Northern river shrimp raised in BFT could be an alternative for the development of small-scale aquaculture in arid zones and feasible areas of Chile.

In biofloc systems, the main advantages when compared to traditional systems are the feed and water savings, as well as improvements on biosecurity. In addition, the *C. caementarius* showed a high tolerance to environmental variables such as suspended solids, ammonium, nitrites, nitrates, alkalinity, hardness, temperature and pH, thus demonstrating as a species with adequate characteristics for cultivation in BFT. On the other hand, research focus in terms of juveniles nursery duration and densities that optimize growth and uniformity is still scarce. A good reference species where this technology has been tested and demonstrated promising results is the giant Malaysian freshwater prawn *Macrobrachium rosenbergii*. Such species belonging to the same family both live in freshwater and share similar territorial behaviour. Some studies have been developed to determine the benefits of BFT on the commercial cultivation of *M. rosenbergii*, evaluating the addition of carbon sources and the nutritional value of biofloc as a food supplement (Asaduzzaman *et al.* 2008; Crab *et al.* 2010a; Pérez-Fuentes *et al.* 2013; Pérez-Rostro *et al.* 2014). Certainly, more research needs to be done to clarify the optimal performance conditions in BFT for freshwater shrimps in different phases.

### Future challenges

Biofloc technology has proven to be an environmentally friendly technology that optimizes the productivity of cultivated species. During the last two decades, research has intensified significantly, but such an increase has not been reflected proportionally at the commercial level. More research is needed to understand the complexity of the biofloc ecosystem (e.g. microbial relations, gut health,

physiological and immune interactions). In addition, in a commercial scale we deal with some complexity in terms of production management and water quality monitoring/interpretations. In this sense, knowledge and skills are still limited and need to be addressed to support the technology.

Another subject that requires further investigation involves to determine the tolerance levels in terms of water quality of new culture species when raised to biofloc technology. In general, the reference tolerance levels derived from conventional systems that use clear water or water exchange and are not necessarily applicable to organisms that are cultivated BFT with zero or limited water exchange and high levels of solids and interacting microbiota. In most cases, scientific investigations are small-scale studies developed under controlled laboratory conditions, in most of the cases far from commercial conditions. The interacting factors are more diverse, thus hindering the technology transfer process and implementation on a larger scale. The inherent gap between these two production scales could explain the disparity of some results. In this sense, it is necessary to scale-up from experimental to commercial conditions. Economic analysis performed on commercial scale is key to determine the cost and feasibility of modules or farm's implementation. High energy demand for adequate aeration, water movement (keep the bioflocs in suspension), pumping and the maintenance of adequate levels of solids certainly limits the BFT system implementation. Alternative energy sources such as solar panels, wind turbines and gas produced through biodigesters are avenues to be considered. Other areas of research are the genetic selection of species or cohorts with better adaptability to intensive or super-intensive biofloc cultivation. Studies are also needed to understand disease resistance (e.g. *Vibrio sp.*) and the application on native species. Exploring the potential of biofloc technology in the shrimp *C. caementarius* will allow the aquaculture development in arid areas and promote a social responsibility with environmental concerns.

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